User Guide

PureProteome™ Nickel Magnetic Beads

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Introduction

The purification of recombinant proteins has been facilitated by the addition of affinity purification tags to the protein sequence. We have developed para-magnetic affinity media for the purification of recombinant, His-tagged proteins based on the well established nickel ion/histidine interaction. Cell lysates prepared under native or denaturing conditions are brought into contact with the PureProteome™ Nickel Magnetic Beads to bind the His-tagged protein. The beads are isolated using a magnetic stand, followed by wash steps to remove unbound proteins. The bound protein is then eluted at a high purity. This magnetic system is a simple and convenient bench-top format capable of purifying up to a milligram of protein. The PureProteome™ Nickel Magnetic Beads can be used for screening or for larger scale purification of His-tagged proteins.

Materials Required

For optimal performance, the PureProteome™ Magnetic Stand is recommended for use with PureProteome™ Nickel Magnetic Beads.

Recommended Buffers

Lysis/Equilibration Buffer

- For Purification Under Native Conditions:
  50 mM sodium phosphate, 300 mM sodium chloride, 0–10 mM imidazole, pH 8
- For Purification Under Denaturing Conditions:
  8 M urea, 100 mM sodium phosphate, 10 mM Tris HCl, pH 8, **or**
- 6 M guanidine-HCl, 100 mM sodium phosphate, pH 8

Wash Buffer

- 50 mM sodium phosphate, 300 mM sodium chloride, 5–20 mM imidazole (typically 10 mM), pH 8

Elution Buffer

- 50 mM sodium phosphate, 300 mM sodium chloride, 300 mM imidazole, pH 8

Note:

- Lysis/Equilibration and Wash Buffers can be supplemented with 0.1% Tween® 20, Triton® X-100, or Nonidet™ P40 detergents.
- When purifying under denaturing conditions, it may be necessary to supplement the Wash and Elution Buffers with either urea or guanidine to prevent protein precipitation.

Sample Preparation Guidelines

This section provides recommendations for sample preparation in order to obtain optimal performance from PureProteome™ Nickel Magnetic Beads.

Selecting Optimal Conditions

PureProteome™ Nickel Magnetic Beads may be used for rapid purification of recombinant His-tagged proteins under native or denatured conditions. Optimal conditions for recombinant protein purification depend on the solubility of the protein being studied. Moreover, depending on the secondary structure of the protein and His-tag availability, some proteins bind to the resin better in a native state while others may bind more optimally in a denatured state. While denaturing conditions allow purification of insoluble protein, biological activity will be lost and the protein must be refolded to restore function.

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To determine whether a recombinant protein is expressed in a soluble form, perform native lysis. After lysate clarification, analyze the protein content of both the supernatant and the pellet by SDS-PAGE and/or western blotting. If the recombinant protein is insoluble, it should be solubilized in urea or guanidine-HCl buffer prior to purification with the PureProteome™ Nickel Magnetic Beads. If guanidine is used for protein solubilization, dilute the lysate to 0.1 M guanidine prior to the addition of SDS loading buffer. Alternatively, urea may be substituted for guanidine.

Preparation of Lysates

Cells should be lysed in buffers compatible with the Nickel Magnetic Bead purification protocol. Reagents such as DTT, SDS, and citrate should be avoided or kept to a minimum (see "Compatibility with Commonly Used Purification Reagents" table). Protease inhibitors (1 mM phenylmethanesulfonyl fluoride [PMSF] or commercially available inhibitors compatible with metal affinity purification) are recommended but should not contain > 5 mM EDTA. Cells can be disrupted by addition of lysozyme, sonication on ice, freeze-thaw cycles, or using a French press or nebulizer. Since lysozyme tends to bind to metal affinity resins, it may contaminate the final preparation if used in concentrations higher than 0.1 mg/mL.

Clarifying Lysates

Clarify cell lysates prior to protein purification by centrifuging at 10,000 × *g* for 20–30 minutes to remove genomic DNA, insoluble proteins, and cell debris. The addition of Benzonase® nuclease to cell lysates will help reduce viscosity and facilitate downstream purification.

Optimizing Binding

By using the appropriate amount of beads, His-tagged protein binding can be maximized while minimizing non-specific protein binding. Typical binding capacity is 1–5.5 µg of recombinant protein per µL of bead suspension. Non-specific binding can be significantly reduced by the addition of low concentrations of imidazole to both the Lysis/Equilibration Buffer (5–10 mM) and Wash Buffer (5–20 mM). Higher concentrations of imidazole may prevent binding of His-tagged proteins. The optimal binding/washing condition for PureProteome™ Nickel Magnetic Beads is at a basic pH (7.5–8), in the presence of 0.3 M NaCl. Refer to the "Recommended Buffers" section for optimized buffer recipes.

Elution

His-tagged proteins can be eluted with 100–500 mM imidazole or low pH. Elution conditions will be dependent on the protein of interest, therefore, optimization will be required.

An eluted protein may be quantified by various protein assay methods. Since most of these methodologies are incompatible with high concentrations of imidazole, removal of the imidazole is necessary. Imidazole removal can be achieved by dilution of the sample, dialysis, or diafiltration. For information on diafiltration, see our Ultrafiltration Application and Product Guide.

The addition of low concentrations of nonionic detergents helps to reduce viscosity, solubilize proteins, and accelerate bead migration to the magnet. We recommend supplementing Lysis/Equilibration and Wash Buffers with 0.1% Tween® 20, Triton® X-100, or Nonidet™ P40 detergents.

Compatibility with Commonly Used Purification Reagents

The chemicals listed below have no significant impact on protein yield. However, compatibility is protein-dependent. Care should be taken when adding new components to the purification buffers.

Reagents		Concentration
Buffers/salts	MOPS, pH 7.4	100 mM
	Tris acetate, pH 7.4	100 mM
	Tris HCl, pH 7.4	100 mM
	HEPES, pH 7.4	100 mM
	NaCl	1 M
Reducing reagents	DTT	5 mM
	Beta-mercaptoethanol	20 mM
Chelating reagents	EDTA	up to 5 mM*
Detergents	Tween® 20 detergent	2%
	Nonidet™ P40 detergent	2%
	Triton® X-100 detergent	2%
	CHAPS	1%
	SDS	Not recommended
Denaturing reagents	Urea	8 M
	Guanidine-HCl	6 M

* Addition of EDTA may slightly reduce protein binding.

Procedure

Nickel Magnetic Beads

This protocol is optimized for 10 mL of bacterial culture or 1 mL of prepared lysate. It may be adjusted to account for varying culture/lysate volumes, as well as for protein expression levels.

1. Resuspend magnetic beads by vortexing.
2. Aliquot 200 µL of magnetic bead suspension (200 µL of PureProteome™ Nickel Magnetic Bead suspension can bind 200–1100 µg of His-tagged protein) into a 1.5 mL microcentrifuge tube.
3. Place the tube into the PureProteome™ Magnetic Stand to collect the beads. Carefully remove storage buffer with a pipette.
4. Resuspend the magnetic beads in 500 µL of Lysis/Equilibration Buffer and incubate with gentle mixing for one minute at room temperature.
5. Place the tube back into the magnetic stand and remove the buffer.
6. Add 1 mL of cell lysate to the magnetic beads and incubate with gentle mixing for 30 minutes at room temperature.
7. Place the tube back into the magnetic stand and allow the beads to migrate to the magnet. With the tube still seated in the magnetic stand, invert to remove residual beads from tube cap. Alternatively, residual liquid can be removed from the cap by brief centrifugation. Capture beads and remove lysate.
8. Wash the magnetic beads by incubating in 500 µL of Wash Buffer with gentle mixing for one minute at room temperature.
9. Place the tube back into the magnetic stand and allow the beads to migrate to the magnet. Remove Wash Buffer.
10. Repeat steps 8 and 9 two more times.
11. Elute the bound protein by adding 100 µL of Elution Buffer. Incubate with gentle mixing for two minutes at room temperature.
12. Place the tube back into the magnetic stand, allow beads to migrate to the magnet, and transfer the eluted fraction into a clean collection tube.
13. Repeat the elution steps 11 and 12 one more time. The first elution will contain the majority of the recombinant protein. If desired, both elutions can be combined, but this will result in an overall lower protein concentration.

One-Step Lysis and Purification Protocol

This His-tag protein purification method allows for the purification of his tagged proteins directly from unclarified lysate. It combines the *E. Coli* lysis step using BugBuster® Master Mix Lysis buffer and protein capture with PureProteome™ Ni Magnetic beads. BugBuster® Master Mix replaces the need for mechanical lysis methods like the French press or sonication. The manual protocol is outlined below but steps can be automated using a bead or liquid handling system.

1. Pellet *E. Coli* culture and discard supernatant.
2. Resuspend pellet in BugBuster® Master Mix and lyse with gentle mixing for of 30 minutes.
3. Resuspend magnetic beads by votexing.
4. Remove the required amount of PureProteome™ Nickel Magnetic Beads and place into appropriately sized tube.
5. Place tube into magnetic stand and allow beads to collect on the magnet. Carefully remove storage buffer.
6. Resuspend the magnetic beads with BugBuster® Master Mix and incubate with gentle mixing for one minute at room temperature.
7. Place tube into magnetic stand and allow beads to collect on the magnet. Carefully remove BugBuster® Master Mix solution.
8. Add the unclarified lysed *E. Coli* (from step 2) to the magnetic beads and incubate with gentle mixing for 30 minutes.
9. Place tube into magnetic stand and allow beads to collect on magnet. Invert the magnetic stand with the tube in place to remove beads left in the tube lid. Carefully remove the lysate. Alternatively, residual liquid can be removed from the cap by brief centrifugation. Capture beads and remove lysate.
10. Resuspend beads in wash buffer and incubate with gentle mixing for 1 minute at room temperature.
11. Place tube into magnetic stand and allow beads to collect on magnet. Carefully remove wash buffer.
12. Repeat steps 10 & 11 two more times.
13. Add elution buffer to the tube and incubate with gentle mixing for two minutes at room temperature.
14. Place tube into magnetic stand and allow beads to collect on the magnet. Carefully remove eluted fraction and place in a clean tube.
15. Repeat steps 13 & 14 one more time. First elution will contain most of the captured recombinant protein. Both fractions may be combined but this will reduce the overall protein concentration.

Troubleshooting/Optimization

Problem	Cause	Solution
Recombinant protein is not present in the eluate	Protein is insoluble (present in inclusion bodies).	After cell lysate clarification by centrifugation, check both supernatant and pellet for the presence of the recombinant protein. Bind under denaturing conditions.
	Protein binds to PureProteome™ Nickel Magnetic Beads tightly and does not elute.	After the binding step, check the lysate for the depletion of the recombinant protein. Increase concentration of imidazole in the elution buffer.
	Recombinant protein is degraded during the process of cell lysis.	Add protease inhibitors to the cell lysate. Perform sonication in short bursts, keeping the tube on ice between bursts.
	pH of the Lysis/Equilibration Buffer is incorrect.	Binding buffer pH should be between 7 and 8. Acidic buffer will inhibit binding.
	SDS or citrate in the Lysis/ Equilibration Buffer prevents binding of His-tagged protein.	Refer to "Compatibility with Commonly Used Purification Reagents" section.
	High concentration of imidazole in the Lysis/Equilibration Buffer.	Do not use more than 5–10 mM imidazole. If the problem persists, use Lysis/Equilibration buffer without imidazole.
	His-tag is not exposed for binding to the affinity resin.	The protein may require denaturing conditions for binding. Express protein with His-tag on the other terminus.
	Protein is not expressed correctly or His-tag is not present.	Sequence the plasmid, check cell lysate for the presence of correctly expressed protein via an independent method (e.g., western blotting).
	Protein induction insufficient.	Confirm that cells have grown to appropriate optical density prior to induction of recombinant protein expression. Make sure appropriate reagents are used for induction of expression.
Magnetic beads do not migrate to the magnet	Magnet strength is not sufficient.	The PureProteome™ Magnetic Stand is required for optimal performance with PureProteome™ Nickel Magnetic Beads.
	Cell lysate is too viscous.	Add nuclease to reduce lysate viscosity. Add 0.1% Tween® 20, Triton® X-100, or Nonidet™ P40 detergent to the cell lysate.
High non-specific binding	Ionic strength of the Lysis/ Equilibration and Wash Buffers is not sufficient.	Adjust salt concentration to at least 0.3 M NaCl. Concentrations up to 1 M NaCl are compatible.
	Binding of non-His-tagged proteins.	Add 5-20 mM imidazole to the Lysis/Equilibration and Wash Buffers. Up to 50 mM imidazole may be needed in some cases.
	Lysozyme contamination of the cell lysate.	Lysozyme tends to bind metal affinity resins. Use lower concentration of lysozyme (0.1 mg/mL), and/or employ other means to disrupt cells (sonication, French Press, freeze-thaw cycles, or a nebulizer).
	Insufficient washing of the beads.	Add wash steps or use a higher volume of Wash Buffer.
	Magnetic beads carry over.	If some magnetic beads are present in the final elution fractions, non-specifically bound proteins may be stripped off in the SDS loading buffer and appear on the gel. Normally, these proteins will not elute with imidazole. Allow longer time for the beads to migrate to the magnet to avoid carry over.
	His-tagged protein is degraded.	Degradation products also bind PureProteome™ Nickel Magnetic Beads and may look like contaminating bands on SDS-PAGE gel. Add protease inhibitors to the cell lysis buffer, perform lysis on ice.

Specifications

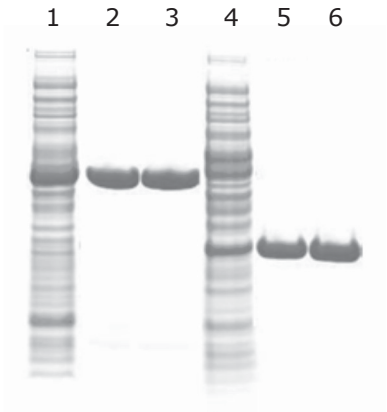
Matrix	Polymer-coated inorganic beads, pre-charged with Ni^{2+}
Particle form	Spherical
Bead diameter	10 µm (nominal)
Storage	2–8 °C. Do not freeze.
Capacity*	Typically 1–5.5 µg of a 30 kDa His-tagged protein per µL of bead suspension
Protein purity*	Typically ≥ 90% by gel densitometry

* Performance will vary depending on the properties of the individual proteins being used.

PureProteome™ Nickel Magnetic Beads are for research use only.

Performance

Purification with PureProteome™ Nickel Magnetic Beads



Replicate purification of 38 kDa (lanes 2, 3) and 24 kDa (lanes 5, 6) His-tagged proteins using PureProteome™ Nickel Magnetic Beads. Lanes 1 and 4, corresponding E.coli cell lysates; lanes 2, 3, 5, 6, eluted recombinant proteins.

Disposal

Used material may be discharged into sewer or industrial waste water systems if allowed by local regulations. Otherwise, collect and dispose according to federal, state, and local regulations.

Safety Data Sheet

Safety Data Sheets (SDS) are available at SigmaAldrich.com.

Product Ordering

Purchase products online at SigmaAldrich.com.

Description	Qty/Pk	Cat. No.
PureProteome™ Nickel Magnetic Beads	2 × 1 mL	LSKMAGH02
	1 × 10 mL	LSKMAGH10
PureProteome™ Magnetic Stand, 8-well	1	LSKMAGS08
PureProteome™ Magnetic Stand, 15 mL	1	LSKMAGS15

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